AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 33, line 26 with the following:

Plasmid construction.

Isolation of plasmid DNA and all other molecular biology procedures were carried out according to standard published procedures. To confirm correct insertion of the desired fragments, plasmids were subjected to double-stranded DNA sequencing ([Sequenase] SEQUENASE 2.0) according to the manufacturer's specifications. PCR primers to the 5' and 3' ends of the *Ipa*C or *Sip*C DNA sequences were produced based on their published sequences. Each 5' primer contained the sequence GAGA (SEQ ID NO: 3), an NdeI restriction site and 18 bases of the 5' end of each gene, respectively. Each 3' primer contained GAGA (SEQ ID NO: 3), a BamHI restriction site and 18 bases of the 3' end of each gene, respectively. Each sequence was amplified by PCR in a standard 100 µl reaction containing 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 100 pmol of the 5' and 3' primers, 10 µl boiled *S. flexneri* or *S. typhimurium*, and 5 U Taq DNA polymerase. Reactions were allowed to proceed in a Perkin-Elmer 480 thermal cycler programmed for 29 cycles (94° C, 45 sec; 63° C, 30 sec; and 72° C, 60 sec) with one additional cycle for 10 min at 72° C. Upon establishing that each PCR product was of the correct size by agarose gel electrophoresis, 7 µl of the reaction mixture was used directly for ligation of the fragment into the pCRII plasmid (Invitrogen, Inc., San Diego, CA) according to manufacturers specifications. The plasmids were then transformed into *E. coli* INVaF' and the transformants containing inserts identified by blue-white screening. The presence of the specific *Ipa*C (SEQ ID NO. 2) or *Sip*C (SEQ ID NO. 1) gene fragments was then confirmed by PCR using the conditions described above (except that 25 µl reactions were used with a T7 promoter forward primer and M13 reverse primer).

Please replace the paragraph beginning on page 36, line 12 with the following:

SDS-PAGE and Western blot analysis.

SDS-PAGE was performed using the standard procedure of Laemmli, *Nature* 227:680, 1970. Following electrophoresis on 9% polyacrylamide gels, the samples could be stained with [Coomassie] COOMASSIE brilliant blue R250 or the proteins electroblotted to PVDF membranes (MSI, Westborough, MA) for Western blot analysis using a [BioRad] BIORAD Transblot Semi-dry Blotter according to the manufacturer's instructions . Western blot analysis was performed. Briefly, the membranes were blocked following protein transfer by incubation in nonfat dry milk in TBS (10 mM Tris-HCl pH 7.5, 150 mM NaCl) and then incubated with anti-SipC polyclonal antibodies or anti-IpaC monoclonal antibodies diluted in TBS containing 1 mM EDTA and 1% NP-40 (*v/v*). After several rinses in the same buffer, the membrane was incubated in ^{125}I-labeled protein G (100,000 dpm/ml) in the same buffer. The membrane was then rinsed in TBS containing 1 mM EDTA, 1 M NaCl and 0.4% N-laurylsarcosine (*w/v*), wrapped in plastic wrap, and exposed to Fuji X-ray medical film.

Please replace the paragraph beginning on page 27, line 10 with the following:

After allowing the production of the recombinant invasin protein in the host cells, the protein is purified according to a protocol appropriate for the affinity purification moiety employed in the method of the present invention, with the modification that all reagent solutions contain a protein denaturant. As the invasin adjuvant should be soluble in the cytosol of the host cell, or in the culture media if secreted, the supernatant should be used in the purification process once the cells or cell lysis debris have been pelleted by centrifugation. At this point, the denaturant should be added to

the protein solution to an appropriate concentration. Preferred denaturants for use in the present invention include guanidine hydrochloride and urea. Although surfactants such as [Tween] TWEEN and [Triton] TRITON may be used in the present invention, they are not preferred because of their tendency to form micelles, which are difficult to remove completely. The most preferable denaturant for use in the present invention is urea because of its efficacy as a denaturant and relatively low toxicity. The appropriate concentration for the denaturant in the protein solution is that concentration which will inhibit protein-protein interactions. For urea, this concentration is preferably between about 1 M and about 10 M, more preferably between about 5 M and about 7 M, and most preferably about 6 M. All solutions used in the purification process most preferably contain a denaturant at an appropriate concentration.

Please replace the paragraph beginning on page 44, line 21 with the following:

ELISA Assay

An ELISA assay was used to measure the levels of IgG subclasses to ovalbumin following immunization. The amount of ovalbumin used in the assay to coat the assay wells was 1 µg/well. Primary antibodies from the blood samples obtained are diluted 1:360 in 2% casein and are incubated with the ovalbumin antigen for 4 hours. After washing in PBS/[Tween] TWEEN 20, plates were probed for 1 hour with monoclonal antibodies against mouse IgG subclasses IgG1, IgG2a, IgG2b, and IgG3 labeled with alkaline phosphatase obtained from Pharmingen, Inc., San Diego, CA. The optical density (O.D.) was measured at 405 nm.

Please replace the paragraph beginning on page 35, line15 with the following:

Affinity column chromatography using [HisBind] HISBIND resin was performed at 4°C according to manufacturer's specifications (Novagen, Madison, WI), except that all buffers were augmented with 6 M urea. Briefly, 5 ml of [HisBind] HISBIND resin in a 10 ml/glass column was washed with 15 ml of water, 25 ml of 50 mM $NiSO_4$ and 15 ml of binding buffer + urea to 6 M. The soluble fraction was passed over the resin and protein that bound nonspecifically was washed from the resin with 50 ml of binding buffer followed by 50 ml of washing buffer (20 mM Tris-HCl pH 7.9, 0.5 M NaCl, 60 mM imidazole) + urea to 6 M. The HisTag-Ipa fusion protein was then eluted from the column with elution buffer (20 mM Tris-HCl pH7.9, 0.5 M NaCl, 1 M imidazole) + urea to 6 M. At each step of the purification process, the concentration of protein in the sample was determined using the bicinchoninic acid (BCA) micro-assay (Sigma Chemical Co., St. Louis, MO) according to the manufacturer's instructions. The samples were stored at -20° C and the [HisBind] HISBIND resin was regenerated with 20 mM Tris-HCl pH 7.9, 0.5 M NaCl, 100 mM EDTA.

Please replace the paragraph beginning on page 37, line 3 with the following:

The recombinant SipC and IpaC fusion proteins described here contain a thrombin cleavage site at the junction between the target protein and its N-terminal HisTag leader. IpaC and SipC do not contain a thrombin cleavage site. Site-specific cleavage of each protein with thrombin yields a native IpaC or SipC protein product with two additional amino acids at its N terminus. After thrombin cleavage, the HisTag-containing leader peptide could be separated from the recombinant Ipa protein product by adding charged [HisBind] HISBIND resin to the mixture and lightly centrifuging to pellet the resin (along with the HisTag leader) while leaving the soluble Ipa protein in the supernatant. Thrombin cleavage efficiency approached completion using an overnight incubation at 20° C.

Please replace the paragraph beginning on page 11, line 19 with the following:

FIG. 1 shows a schematic of a linearized plasmid pET15b containing a DNA sequence encoding a recombinant invasin protein (IpaC or SipC). SEQ. ID. NO 19 (GAGACATATG) and SEQ. ID. NO. 20 (GGATCCGAGA) are also depicted.